1: J Protein Chem. 1999 Aug;18(6):619-25. Related Articles, L

Oxidative folding of reduced and denatured huwentoxin-I.

Liang S, Shu Q, Wang X, Zong X.

College of Life Sciences, Hunan Normal University, Changsha, China.

Huwentoxin-I, a neurotoxic peptide with 33 amino acid residues and three disulfide bond was used to investigate the pathway of reduction/denaturation and of oxidative folding in small proteins with multiple disulfide bonds. Titration of thiol groups, reversed-phase HPLC, 1D NMR spectroscopy, and biological activity assays were used to monitor the extent of reduction/ denaturation and renaturation of the toxin. The reduction and denaturation of huwentoxin-I resulted in a 100% loss of bioactivity as measured in a mo phrenic nerve-diaphragm preparation. About 90% of full biological activity could be restored under optimized conditions of oxidative refolding of the reduced peptide. Sever reaction conditions employing air oxidation, oxidized and reduced glutathione (GSSG an GSH), and cystine/cysteine were investigated in order to find optimal conditions for renaturation of huwentoxin-I. The best renaturation yield was achieved in 0.1 mM GSSG and 1 mM GSH at pH 8.5 and 4 degrees C over 24 hr. High concentrations of glutathion and high temperatures reduced renaturation yields. Oxidative refolding of huwentoxin-I air requires about 6 days for maximal yields and is inhibited by EDTA.

PMID: 10609637 [PubMed - indexed for MEDLINE]

h cb h g e e e fcg c e e e b b e

1: P83591. Hainantoxin-I (Hn...[gi:32363268] BLink, L

```
LOCUS       P83591                    33 aa            linear   INV 15-SEP-2003
DEFINITION  Hainantoxin-I (HnTx-I).
ACCESSION   P83591
VERSION     P83591  GI:32363268
DBSOURCE    swissprot: locus TXN1_SELHA, accession P83591;
            class: standard.
            created: Sep 15, 2003.
            sequence updated: Sep 15, 2003.
            annotation updated: Sep 15, 2003.
KEYWORDS    Toxin; Neurotoxin; Ionic channel inhibitor; Sodium channel
            inhibitor; Amidation.
SOURCE      Ornithoctonus hainana
  ORGANISM  Ornithoctonus hainana
            Eukaryota; Metazoa; Arthropoda; Chelicerata; Arachnida; Araneae;
            Mygalomorphae; Theraphosidae; Ornithoctonus.
REFERENCE   1  (residues 1 to 33)
  AUTHORS   Li,D.-L., Xiao,Y.-C. and Liang,S.-P.
  TITLE     Direct Submission
  JOURNAL   Submitted (~MAY-2003) to Swiss-Prot
  REMARK    SEQUENCE, FUNCTION, SUBUNIT, SUBCELLULAR LOCATION, TISSUE
            SPECIFICITY, MASS SPECTROMETRY, DISULFIDE BONDS, AMIDATION, AND
            STRUCTURE BY NMR.
            TISSUE=Venom
COMMENT     [FUNCTION] Is a depressant toxin. Binds and blocks insect sodium
            channels without altering the activation or inactivation kinetics.
            [SUBUNIT] Monomer.
            [SUBCELLULAR LOCATION] Secreted.
            [TISSUE SPECIFICITY] Expressed by the venom gland.
            [MASS SPECTROMETRY] MW=3608.01; METHOD=MALDI.
            [SIMILARITY] Belongs to the huwentoxin-I family.
FEATURES             Location/Qualifiers
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                     /organism="Ornithoctonus hainana"
                     /db_xref="taxon:209901"
     Protein         1..33
                     /product="Hainantoxin-I"
     Bond            bond(2,17)
                     /bond_type="disulfide"
     Bond            bond(9,22)
                     /bond_type="disulfide"
     Bond            bond(16,29)
                     /bond_type="disulfide"
     Site            33
                     /site_type="amidation"
ORIGIN
        1 eckgfgkscv pgkneccsgy acnsrdkwck vll
//
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h cb h g e e e e fcg b e

1: Biochemistry. 1992 Feb 18;31(6):1749-56.

Disulfide exchange folding of insulin-like growth factor I.

Hober S, Forsberg G, Palm G, Hartmanis M, Nilsson B.

Department of Biochemistry and Biotechnology, Royal Institute of Technology, Stockholm, Sweden.

The disulfide exchange folding properties of insulin-like growth factor I (IGF-I) have be analyzed in a redox buffer containing reduced (10 mM) and oxidized (1 mM) glutathione Under these conditions, the 3 disulfide bridges of the 70 amino acid peptide were not quantitatively formed. Instead, five major forms of IGF-I were detected, and these components were concluded to be in equilibrium as their relative amounts were similar starting from either reduced, native, or a mismatched variant of IGF-I containing two no native disulfides. The different components in the mixtures were trapped by thiol alkylat using vinylpyridine and subsequently isolated by reverse-phase HPLC. The purified variants were further characterized using plasma desorption mass spectrometry and pept mapping. Two of the five different forms were identified as native and mismatched IGF- One form was a variant with only one disulfide bond, and the other two major componen had two disulfides formed. In a separate experiment, early refolding intermediates were trapped by pyridylethylation after only 90 s of refolding in the glutathione buffer, startin from reduced IGF-I. The intermediates were identical to the components observed at equilibrium, but at different relative concentrations. On the basis of the disulfide bond patterns of the different components in the equilibrium mixtures, we conclude that the disulfide between cysteines-47 and -52 in IGF-I is an unfavorable high-energy bond that may exist in the native molecule in a strained configuration.

PMID: 1737028 [PubMed - indexed for MEDLINE]

h cb h g e e e fcg c e e e b b e b

1: Protein Sci. 1999 Aug;8(8):1605-13.

The disulfide-coupled folding pathway of apamin as derived from diselenide-quenched analogs and intermediates.

Pegoraro S, Fiori S, Cramer J, Rudolph-Bohner S, Moroder L.

Max-Planck-Institut fur Biochemie, Martinsried, Germany.

The sequence of apamin, an 18 residue bee venom toxin, encloses all the information required for the correct disulfide-coupled folding into the cystine-stabilized alpha-helica motif. Three apamin analogs, each containing a pair of selenocysteine residues replacing the related cysteines, were synthesized to mimic the three possible apamin isomers with two crossed, parallel, or consecutive disulfides, respectively. Refolding experiments clea revealed that the redox potential of selenocysteine prevails over the sequence encoded structural information for proper folding of apamin. Thus, selenocysteine can be used as new device to generate productive and nonproductive folding intermediates of peptides a proteins. In fact, disulfides are selectively reduced in presence of the diselenide and the conformational features derived from these intermediates as well as from the three-dimensional (3D) structures of the selenocysteine-containing analogs with their nonnatu networks of diselenide/disulfide bridges allowed to gain further insight into the subtle driving forces for the correct folding of apamin that mainly derive from local conformational preferences.

PMID: 10452604 [PubMed - indexed for MEDLINE]

h cb h g e e e fcg c e e e b b e

1: Arch Biochem Biophys. 1998 Jun 1;354(1):1-8.

Unfolding/folding studies on cobrotoxin from Taiwan cobra venom: pH an GSH/GSSG govern disulfide isomerization at the C-terminus.

Chang LS, Lin SR, Chang CC.

Department of Biochemistry, Kaohsiung Medical College, Taiwan, Republic of China. lschang@mail.nsysu.edu.tw

Refolding of cobrotoxin was assessed by the exposure degree of its single Trp determine by an acrylamide quenching study. The change in the accessibility of Trp for acrylamide quantitatively reflected the formation of folded cobrotoxin, and the data were confirmed HPLC and gel electrophoresis analyses. However, the site-specific information provided quenching Trp fluorescence revealed that the ordered structure in the neighborhood of T was attained prior to the complete formation of the tertiary structure of cobrotoxin. HPL analyses showed that, in addition to refolded cobrotoxin, two novel species (cobrotoxin I and cobrotoxin III) with isomerization of disulfide bonds at the C-terminus of the toxin molecule were produced along the folding reaction. The disulfide pairings in cobrotoxin and cobrotoxin III were Cys43-Cys55 and Cys54-Cys60 and Cys43-Cys60 and Cys54-Cys55, respectively. Among the three possible two-disulfide species at the C-terminus, t disulfide linkages Cys43-Cys60 and Cys54-Cys55 of cobrotoxin III caused a marked decrease in lethality and resulted in a conformation which was notably different from tha observed with the native toxin molecule as evidenced by CD spectra. The refolding reaction was accelerated by the addition of GSH/GSSG, and the resulting products were mostly folded cobrotoxin. However, if GSH/GSSG was not added into the initial folding materials, the yields of cobrotoxin II and cobrotoxin III greatly increased. The conversio of cobrotoxin to its isomers was to be irreversible and pH-dependent: the higher the pH, faster the rate of conversion. However, this conversion could be partly inhibited by GSH/GSSG. Cobrotoxin II and cobrotoxin III were purified from Taiwan cobra venom a well, and their yields in comparison to that of cobrotoxin in venom were similar to that noted with the folded products in the presence of GSH/GSSG. Moreover, the rate of disulfide isomerization was expected to be slow in venom fluid in which the pH was approximately pH 6.2. Thus, the finding that cobrotoxin represents the predominant neurotoxin species in Taiwan cobra venom is probably associated with the synergistic effects of GSH/GSSG and pH.

PMID: 9633591 [PubMed - indexed for MEDLINE]

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h cb h g e e e fcg c e e e b b e b

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Refolding by disulfide isomerization: the mixed disulfide between ribonuclease T1 and glutathione as a model refolding substrate.
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Synthesis, bioactivity, and cloning of the L-type calcium channel blocker omega-conotoxin TxVII.
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Analogies and differences between omega-conotoxins MVIIC and MVIID: binding sit and functions in bovine chromaffin cells.
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Tyr13 is essential for the activity of omega-conotoxin MVIIA and GVIA, specific N-type calcium channel blockers.
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A molecular mechanism for toxin block in N-type calcium channels.
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h cb h g e e e fcg ge b e

PMID: 9605543 [PubMed - indexed for MEDLINE]

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The disulfide-coupled folding pathway of apamin as derived from diselenide-quenched analogs and intermediates.
Protein Sci. 1999 Aug;8(8):1605-13.
PMID: 10452604 [PubMed - indexed for MEDLINE]

☐ **59:** Chang LS, Lin SR, Chang CC. Related Articles, Li

Unfolding/folding studies on cobrotoxin from Taiwan cobra venom: pH and GSH/GSS govern disulfide isomerization at the C-terminus.
Arch Biochem Biophys. 1998 Jun 1;354(1):1-8.
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Solution structure of the calcium channel antagonist omega-conotoxin GVIA.
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h cb h g e e e fcg ge b e

1: J Pept Res. 2003 Apr;61(4):202-12.

Detergent-assisted oxidative folding of delta-conotoxins.

DeLa Cruz R, Whitby FG, Buczek O, Bulaj G.

Department of Biology, University of Utah, Salt Lake City, Utah 84112, USA.

Conotoxins comprise a diverse group of disulfide-rich peptides found in venoms of predatory Conus species. The native conformation of these peptides is marginally stable comparison with alternative conformations, often resulting in low folding yields. The oxidative folding of hydrophobic delta-conotoxins was found to produce less than 1% of the native peptide [Bulaj, G. et al. (2001) Biochemistry 40, 13201]. In order to identify factors that might improve folding yields, we screened a number of additives including water-soluble polymers, detergents and osmolytes for their ability to increase steady-stat accumulation of the native delta-conotoxin PVIA. The presence of a non-ionic detergent Tween and low temperature appeared to be the most effective factors in improving the oxidative folding. The detergent was also effective in promoting folding of other hydrophobic delta-conotoxins. Based on our findings, we discuss a possible mechanism detergent-assisted folding and the general applicability of this mechanism to facilitating proper folding of hydrophobic, cysteine-rich peptides.

PMID: 12605605 [PubMed - indexed for MEDLINE]

h cb h g e e e fcg c e e e b b e

1: Biopolymers. 1996 Jun;38(6):733-44. Related Articles, L

Oxidative folding of omega-conotoxin MVIIC: effects of temperature and salt.

Kubo S, Chino N, Kimura T, Sakakibara S.

Peptide Institute, Inc., Protein Research Foundation, Osaka, Japan.

Oxidative folding of omega-conotoxin MVIIC, a highly basic 26-amino acid peptide wit three disulfide bonds, predominantly gave two products with mismatched disulfide bond in 0.1M NH4OAc buffer (pH 7.7) at 21 degrees C both in the presence and absence of redox reagents such as reduced and oxidized glutathione. A low reaction temperature (5 degrees C) and a high salt concentration in buffer such as 2M (NH4)2SO4 were necessar to obtain the correctly folded biologically active product. The folding reaction was found proceed via a two-stage pathway of (I) the formation and (II) the rearrangement of the mismatched disulfide bonds. Both the reaction temperature and the salt strongly affected the equilibrium between mismatched and correctly formed disulfide bonds in the second stage. Such an effect of salts on the rearrangement reaction could be explained by anion binding at a low concentration and the salting out effect at a high concentration by analyzing the rank order of their effectiveness. The anion-binding effect was also confirmed by examining the folding of the tetra-acetylated peptide at the Lys side chains CD study suggested that the yield of the biologically active product was correlated with conformational change as functions of temperature and salt concentration.

PMID: 8652794 [PubMed - indexed for MEDLINE]

h cb h g e e e fcg c e e e b b e b